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Patterns of mRNA prevalence and expression of B1 and B2 transcripts in early mouse embryos

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Summary

Considerable evidence indicates that the 2-cell stage is a critical period of mouse embryo development when a transition from maternal to zygotic genomic control takes place. The overall changes in the structure of the mRNA population as a result of this transition were explored using a random cDNA library of 69 clones derived from late 2-cell embryos. The prevalence of the cloned sequences was analysed by dot hybridization of the cDNA clones with labelled cDNA probes synthesized to poly(A)$^+$ RNA from different stages of development from 1-cell through blastocyst. The number of copies of individual transcripts was quantitatively estimated by comparison to standard clones of known prevalence. About one half of the transcripts that gave a measurable reaction at the 2-cell and later stages were not represented detectably in egg RNA, suggesting that a large set of zygote-specific genes not included in the maternal gene set becomes transcriptionally active in the 2-cell embryo. Six of the cDNA clones represented B1 and B2 repeat sequences. As measured by hybridization with labelled cDNA, B1 and B2 transcripts were abundantly expressed throughout cleavage, being represented by about 10^5 to 10^6 copies per embryo. However, the developmental pattern of prevalence was different for the two transcripts suggesting that their expression is regulated independently. The results of this study corroborate previous evidence derived from protein synthetic patterns and *in vitro* translation experiments that a major qualitative shift in the mRNA population occurs in the 2-cell embryo.

Key words: mouse embryo, mRNA, B1 and B2 repeats, cDNA clones.

Introduction

In the early mouse embryo, there is now considerable information on the period of development dependent on maternally derived gene transcripts and the initiation of transcriptional activity by the embryonic genome. The ovulated mouse egg contains about 350 pg total RNA of which about 8–10 % is polyadenylated (Bachvarova & De Leon, 1980; Pikó & Clegg, 1982; Bachvarova, 1985). As the 1-cell zygote synthesizes only a very low level of poly(A)$^+$ RNA and no detectable amounts of ribosomal RNA (Clegg & Pikó, 1982, 1983*b*), it is clear that protein synthesis through the first cleavage is supported mostly, if not entirely, by maternally inherited components. Some characteristic changes in the protein synthetic pattern during this period could be attributed, in part, to the selective utilization of stored maternal mRNA (Braude, Pelham, Flach & Lobatto, 1979; Cascio & Wassarman, 1982) and, in part, to post-translational modifications (Van Blerkom, 1981; Howlett & Bolton, 1985; Howlett, 1986). The recruitment of maternal mRNA is suggested also by the high rate of cytoplasmic polyadenylation of previously nonpolyadenylated RNA in the 1-cell embryo (Clegg & Pikó, 1983*a*,*b*).

Several lines of evidence indicate that a transition from maternal to zygotic genomic control occurs during the 2-cell stage of development. By the late 2-cell stage, about 40 % of the bulk maternal RNA is degraded (Bachvarova & De Leon, 1980) and the number of polyadenylated RNA molecules is reduced to about one third as compared to that in the egg, suggesting a large-scale elimination of the stored

maternal mRNA (Pikó & Clegg, 1982; Clegg & Pikó, 1983*a*). During the same period, the maternal stores of the mRNAs coding for actin and several histones decline by as much as tenfold (Giebelhaus, Heikkila & Schultz, 1983; Giebelhaus, Weitlauf & Schultz, 1985; Graves, Marzluff, Giebelhaus & Schultz, 1985). In the 2-cell embryo, all the major classes of RNA are synthesized (Clegg & Pikó, 1982), and the increased RNA synthetic activity coincides with marked changes in the qualitative pattern of protein synthesis (Van Blerkom & Brockway, 1975; Levinson, Goodfellow, Vadeboncoeur & McDevitt, 1978; Howe & Solter, 1979; Cullen, Emigholz & Monahan, 1980); the product of a paternally derived gene (β_2-microglobulin) has been detected at this time (Sawicki, Magnuson & Epstein, 1981) and a regulatory mechanism controlled by the paternal genome seems to be active by the late 2-cell stage (Szöllösi & Yotsuyanagi, 1985). A detailed study of the sequence of α-amanitin-sensitive changes in the protein synthetic pattern suggests two separate transcriptional events: one occurs immediately after the first cleavage and results, in 2–4 h, in the appearance of a major polypeptide band of about $68\times10^3 M_r$; the second phase of transcription begins about 6 h after cleavage and results in a pronounced change in the protein synthetic profile involving many polypeptides from the mid-2-cell stage onwards (Flach *et al.* 1982; Bolton, Oades & Johnson, 1984). Bensaude, Babinet, Morange & Jacob (1983) confirmed these findings and showed that the early 2-cell-specific-proteins were identical to two of the mouse heat-shock proteins, hsp68 and hsp70. Between the late 2-cell and early blastocyst (32-cell) stages, both the total RNA content and the number of poly(A)$^+$ RNA molecules increase about fivefold due to a high rate of embryonic RNA synthesis (Clegg & Pikó, 1977, 1983*a*,*b*; Levey, Stull & Brinster, 1978; Pikó & Clegg, 1982). There is a concomitant six- to eightfold increase in the overall rate of protein synthesis (Brinster, Wiebold & Brunner, 1976), but the qualitative pattern of protein synthesis changes only slightly during this period.

The purpose of this study was to explore the qualitative and quantitative changes in the mRNA population as a result of the transition from maternal to embryonic control. A random cDNA library of 69 clones was isolated from late 2-cell embryos and the prevalence of the corresponding transcripts analysed by dot hybridization of the cloned cDNAs with labelled cDNA probes synthesized to poly(A)$^+$ RNA from different stages of development. About one half of the cloned sequences that were expressed from the 2-cell stage onward were not represented detectably in egg RNA, suggesting that a large set of zygote-specific genes not included in the maternal gene set becomes transcriptionally active in the 2-cell embryo. The data also indicate an abundant expression of the B1 and B2 repeat families (mouse Alu-like sequences) in the early mouse embryo.

Materials and methods

Recovery of embryos

Recovery and culture of unfertilized eggs and early embryos followed the general procedures described by Pikó & Clegg (1982). Prepubertal female $CD2F_1$ mice (BALB/c ♀ × DBA/2 ♂; Simonsen Laboratories) were superovulated and mated to $CD2F_1$ males. Eggs and embryos were recovered by flushing the oviducts with Brinster's pyruvate–lactate medium (PLM) at the following intervals after injection of human chorionic gonadotropin (HCG): 20–22 h for unfertilized eggs; 47–49 h for 2-cell embryos; 69–71 h for 8-cell embryos; and 92–94 h for early blastocysts (32 cells). Ovulation occurs at 12–14 h, first cleavage at 30–32 h, and second cleavage at 51–53 h after the injection of HCG. Follicle cells were removed from unfertilized eggs by extensive washes in PLM with hyaluronidase. Batches of eggs and embryos were washed in four changes of Hepes-buffered saline, pH 7·3, transferred into 300 μl NTE buffer (0·1 M-NaCl, 0·05 M-Tris–HCl, pH 7·5, 0·005 M-EDTA) containing 0·1 % SDS and 200 μg ml^{-1} proteinase K (Bethesda Research Laboratories), and kept frozen at −20°C until use.

Extraction of RNA

Poly(A)$^+$ RNA to be used for the synthesis of double-stranded cDNA was isolated from 2-cell embryos as follows. A frozen–thawed sample of about 800 embryos in lysis buffer was incubated at 37°C for 30 min and extracted with an equal volume of phenol:chloroform:isoamyl alcohol (50:48:2) saturated with NTE buffer. The water phase was brought to 0·5 M-NaCl and the poly(A)$^+$ RNA was isolated by binding to poly(U)–Sepharose (Pharmacia) beads (dry weight 3 mg) in suspension as described (Clegg & Pikó, 1982). The beads were washed four times with 0·5 ml binding buffer each and the bound RNA was eluted by six washes, 30 μl each, with 0·01 M-Tris–HCl, pH 7·5, 0·001 M-EDTA at 50°C. The eluate was adjusted to 0·1 M in NaCl and the RNA was ethanol precipitated in the presence of 6 μg MS2 bacteriophage RNA (Boehringer–Mannheim) as carrier. For the isolation of RNA to be used for the synthesis of labelled cDNA probes, batches of about 2000 eggs or embryos were extracted with phenol–chloroform as above, and a total nucleic acid fraction was isolated by passage of the water phase through a Nensorb-20 column (DuPont NEN) as follows. The water phase was extracted six times with water-saturated ether, and traces of ether were removed by blowing a nitrogen stream over the solution for 30 min. An equal volume of buffer A (0·1 M-Tris–HCl, 0·01 M-triethylamine, 0·001 M-EDTA, pH 7·7) was then added, and the solution was passed through the Nensorb column. The column was washed with 3 ml buffer A, followed by two washes with 3 ml each of 0·01 M-Tris–HCl, pH 7·5, 0·1 mM-EDTA, and the nucleic acids were eluted with 0·5 ml of 20 % ethanol. Recovery was about 80 % as monitored by adding 1 ng of lambda

^{3}H-cRNA (specific activity, $2{\cdot}5\times10^7$ cts min^{-1} μg^{-1}) to the original embryo lysate and determining the radioactivity of a sample of the eluate. The eluate was dried in a SpeedVac (Savant) centrifuge under vacuum and redissolved in 100 μl distilled water; it was estimated that this solution also contained about 0·02 M-Tris–HCl and 0·2 mM-EDTA derived from the void volume of the column. The solution was adjusted to 0·01 M-$MgCl_2$, 0·01 M-dithiothreitol, 1000 units ml^{-1} RNasin (Promega) and incubated with 20 μg ml^{-1} DNase I (RNase-free, Worthington) at 37°C for 15 min. Proteinase K was added to 50 μg ml^{-1} and incubation continued for 15 min. After extraction with an equal volume of chloroform, the solution was kept frozen at −70°C until use.

Synthesis and cloning of cDNA

Ethanol-precipitated poly(A)$^+$ RNA from 800 2-cell embryos plus 6 μg MS2 RNA carrier (see above) was collected by centrifugation in a Beckman SW56 rotor at 30000 rev. min^{-1} for 30 min, washed twice with 70 % ethanol and once with 100 % ethanol, and air-dried. The pellet was redissolved in reaction buffer and first-strand synthesis was carried out in a volume of 25 μl in the standard reverse transcriptase reaction (Maniatis, Fritch & Sambrook, 1982). The final reaction mixture contained about 5 ng of embryo poly(A)$^+$ RNA (assuming 80 % recovery; Clegg & Pikó, 1983*a*), 0·1 mM-[^{3}H]dTTP (specific activity, 10 Ci mM^{-1}; Amersham), 1 mM each of dATP, dGTP and dCTP, 80 μg ml^{-1} oligo(dT)$_{12-18}$ primer, 1000 units ml^{-1} RNasin, 1500 units ml^{-1} of avian myeloblastosis virus (AMV) reverse transcriptase (a gift of J. W. Beard, Life Sciences), 0·1 M-KCl, 0·072 M-Tris–HCl, pH 8·3, 7·2 mM-$MgCl_2$, 4·3 mM-dithiothreitol; in addition, 0·032 M-KPO_4, 0·03 % Triton X-100 and 8 % glycerol was contributed by the reverse transcriptase preparation. After incubation at 42°C for 30 min, the reaction was terminated with excess EDTA and the mixture was extracted with an equal volume of phenol–chloroform. The organic phase was reextracted with 25 μl TE buffer (10 mM-Tris–HCl, pH 7·5, 1 mM-EDTA), the water phases were pooled and extracted once with chloroform and once with ether. The cDNA yield and the recovery of cDNA during the various reaction steps was monitored by absorption of a sample to Whatman DE81 paper and scintillation counting. The cDNA–RNA hybrid was precipitated with 0·5 % cetyltrimethylammonium bromide (CTAB) and washed three times with 0·1 % CTAB in 0·1 M-NaOAc, pH 5, and six times with 70 % ethanol:30 % 0·1 M-NaOAc as described (Clegg & Pikó, 1982). After a final wash with 100 % ethanol, the pellet was dried and redissolved in reaction buffer for second-strand synthesis. Second-strand cDNA synthesis, involving the combined activities of *E. coli* RNase H (Pharmacia), *E. coli* DNA polymerase I (Pharmacia) and *E. coli* DNA ligase (New England Biolabs), was carried out in a 25 μl volume according to the procedures of Gubler & Hoffman (1983). Upon termination of the reaction, the sample was extracted with phenol–chloroform and the cDNA was precipitated with CTAB as above. The double-stranded cDNA was dG-tailed (to a length of about 20 residues; Peacock, McIver & Monahan, 1981) using calf thymus deoxynucleotidyl terminal transferase (Bethesda Research Laboratories), annealed with dC-tailed (at the *Hin*cII site) pUC8 plasmid vector, and used to transform *E. coli* JM83 host cells according to the procedures of Hanahan (1983). (The pUC8-JM83 system was obtained from Bethesda Research Laboratories.) Transformed (colourless) colonies were picked for screening by colony hybridization (Maniatis *et al.* 1982) with MS2 probes labelled with [γ-^{32}P]ATP using polynucleotide kinase.

Isolation of plasmid DNA

Clones that did not react with the MS2 probe in preliminary screenings were grown overnight in 10 ml minicultures and used for plasmid isolation according to the preparative method of Birnboim (1983). After treatment with ribonucleases A and T_1, the plasmid DNA was precipitated with CTAB as described above, redissolved in 10 mM-Tris–HCl, pH 7·5, 0·1 mM-EDTA, and kept frozen at −20°C. This procedure yielded 20–40 μg of plasmid DNA, on the basis of absorption at 260 nm. Plasmid DNA was characterized by electrophoresis in 1·5–2 % agarose gels as to purity and appeared to be 80–90 % pure, with only a minor amount of low-molecular-weight material. The size of the insert was measured in these gels after excision with restriction endonuclease cuts on each side of the cloning site. Recombinant plasmids used for subcloning or sequencing were further purified by buoyant density centrifugation in CsCl–ethidium bromide gradients.

Synthesis of cDNA probes for library screening

^{32}P-labelled cDNA for library screening was synthesized in an oligo(dT)-primed reaction as described above for first-strand synthesis but the reaction mixture included 4 μM-[α-^{32}P]dTTP, 1000 Ci mmol^{-1} (ICN), to give a cDNA specific activity of about 2×10^9 cts min^{-1} μg^{-1}, and total carrier-free RNA equivalent to 150 to 600 embryos. The number of embryos was adjusted so that each batch of RNA contained 5–6 ng of total poly(A)$^+$ RNA as calculated from Clegg & Pikó (1983*a*). Upon completion of the reaction, the samples were brought to 0·2 M-NaOH and heated for 5 min in a boiling water bath to hydrolyse the RNA. The solution was neutralized with 1 M-HCl and the cDNA purified by passage through a Nensorb-20 column (DuPont NEN).

Screening of cDNA clones by dot hybridization

Recombinant plasmids were screened with ^{32}P-labelled cDNA probes by a dot hybridization procedure similar to that described previously (Pikó, Hammons & Taylor, 1984). Linearized plasmid DNA was denatured by heating at 65°C for 10 min in 0·025 M-sodium phosphate, pH 7, 0·005 M-EDTA, 2·2 M-formaldehyde, 50 % formamide (deionized), brought to 10 × SSC (1× is 0·15 M-NaCl, 0·015 M-sodium citrate) in a volume of 150 μl, and dotted onto nitrocellulose filters (BA85, Schleicher & Schuell) in a Hybri-Slot apparatus (Bethesda Research Laboratories) in an amount of 200 ng DNA per dot. Preliminary experiments with increasing amounts of plasmid DNA containing MS2 sequences indicated that the rate of hybridization with end-labelled MS2 RNA in solution reached a plateau at about 50 ng DNA per dot. During the dotting procedure, the filter was moved so that the number of slots per filter was doubled. The filters were baked at 80°C for 2 h and

prehybridized for 2 h at 37°C in 1 M-NaCl, 0·05 M-sodium phosphate, pH 7, 0·01 M-EDTA, 0·1 % SDS and 10× Denhardt's solution (1× is 0·02 % each of bovine serum albumin, Ficoll and polyvinylpirrolidone). Prehybridization continued for 1 h at 42°C in the same buffer but containing 1× Denhardt's, 200 $\mu g\,ml^{-1}$ sonicated denatured herring sperm DNA, 20 $\mu g\,ml^{-1}$ poly(A) and 50 % (vol/vol) formamide. ^{32}P-labelled cDNA probe (see below) was then added to $0{\cdot}7–1{\cdot}0\times10^{7}$ cts $min^{-1}\,ml^{-1}$ and hybridization continued in sealed plastic bags for 18 h at 42°C. Each bag contained four pieces of filter, 2·5×5·6 cm each, in 0·7 ml of hybridization mixture. After hybridization, the filters were washed over a period of 2 h at 68°C in several changes of 0·25 M-NaCl, 12·5 mM-sodium phosphate, pH 7, 4 mM-EDTA, 0·1 % SDS, dried and autoradiographed at −70°C on preflashed X-ray films (DuPont Cronex no. 4) with intensifying screens. Multiple exposures were taken from 6 h to 4 days in order to bring the densities of the dots within the linear range of film response. The density of the dots was determined by scanning the autoradiographs with a Biorad Model 1650 densitometer.

Standard clones

Four cloned sequences whose prevalence in early mouse embryos has been determined in independent experiments were included in the cDNA library screening to aid in the quantification of the unknown sequences. (1) A 0·8 kb *Hin*dIII fragment of the intracisternal A-particle (IAP) genome containing the 3′ LTR and adjacent IAP sequences in pUC plasmid (Pikó *et al.* 1984). (2) A 2·3 kb fragment of mouse mitochondrial DNA (mtDNA) containing a 1·41 kb portion of the gene for subunit I (COI) and the entire 0·69 kb gene for subunit II (COII) of cytochrome *c* oxidase in pUC plasmid (Pikó & Taylor, 1987). (3) Clones containing a 0·46 kb fragment of the 3′ untranslated region (UTR) of human fibroblast β-actin mRNA and a 0·54 kb fragment of the 3′ UTR of human fibroblast γ-actin mRNA, respectively, in the plasmid pBR322 (Ponte, Gunning, Blau & Kedes, 1983). We are indebted to Drs P. Gunning and L. Kedes for providing us with these clones. All library screening experiments included pUC DNA as background control.

DNA sequencing

Sequencing of cDNA inserts was carried out either by the dideoxy procedure (according to the protocol provided by Bethesda Research Laboratories), after subcloning the inserts into M13 vectors, or by the chemical degradation method of Maxam & Gilbert (1980). Some clones could not be sequenced by the dideoxy procedure, possibly because of artifacts introduced by the G-C homopolymer tails (Martin, 1987). The final sequence was derived by sequencing both strands. Sequence comparisons were made by searching the European Molecular Biology Laboratory gene library using the ALIGN option of Version 3.2 of the Intelligenetics SEQ program.

Results

Selection of cDNA clones

The cDNA cloning procedure yielded about 1200 transformant colonies, recognizable by their white colour on X-gal plus ampicillin plates. Of these, 960 colonies were isolated singly by toothpicking and confirmed as positive, white clones. Because of the relatively large amount of MS2 carrier RNA used, it was expected that a significant fraction of the clones may have been derived from MS2 RNA. In colony hybridization experiments, 490 clones out of the 960 tested gave a positive reaction with labelled MS2 RNA. Out of the remaining clones, plasmid DNA was isolated from 200 clones and checked for insert size by agarose gel electrophoresis. A subpopulation of 150 clones was estimated to have insert sizes greater than about 0·2 kb and was checked in detail for the reactivity of the insert DNA either in dot hybridization experiments (with end-labelled MS2 RNA and nick-translated mtDNA probes) or by Southern blot hybridization after restriction cleavage of the clones (with nick-translated *E. coli* strain JM83 DNA and nick-translated pUC DNA as probes). In these tests, 63 clones reacted with MS2 RNA, 10 with *E. coli* DNA, one with mtDNA, none of the inserts reacted with pUC DNA, and 76 of the clones were nonreactive with any of these probes. The 76 negative clones ranged in size from about 0·2 kb to 2·0 kb, with a number-average size of 0·35 kb (including the G-C tails), and represented an essentially random sample of the clones that did not react in the preliminary screenings, except for the omission of clones carrying inserts smaller than about 0·2 kb.

Screening of selected clones with embryo-derived cDNA probes

The 76 cDNA clones selected on the basis of the above criteria were screened in dot hybridization experiments with ^{32}P-labelled cDNA probes made to RNA from unfertilized eggs, 2-cell and 8-cell embryos, and early blastocysts. As illustrated in Fig. 1, a wide range of reactivity was observed, from no detectable reaction to a high level of labelling of individual clones. Since we have used carrier-free embryonic RNA for the synthesis of the cDNA probes, we had assumed initially that all the reactive clones represented sequences complementary to embryo RNA. However, further analysis revealed that an additional source of template was provided by the AMV reverse transcriptase preparation itself. The nucleotide sequences of clones A6 and C16 (clones are referred to according to their grid position in Fig. 1), which were highly represented in the cDNA probes from all four stages examined, proved to be 98 % homologous to overlapping 3′ segments of avian

myeloblastosis virus RNA (Klempnauer, Gonda & Bishop, 1982; data not shown). Therefore, an identical set of cDNA clones was screened with a ^{32}P-labelled cDNA probe synthesized by the same AMV reverse transcriptase preparation (which was used throughout this study) but without any added template (data not shown). In addition to clones A6 and C16, five additional clones showed a positive reaction: C10, C13, C17, C22 and C23 (marked with an asterisk in Fig. 1). As might be expected, the reactivity of these clones is about the same in the four experiments shown in Fig. 1, except for clone C10 which reacted more strongly with the one-cell probe. These clones are excluded from further consideration. The reactivity of the remaining 69 clones is assumed to be due to embryo-derived cDNA sequences.

The autoradiographs shown in Fig. 1 derive from a set of experiments that were carried out in parallel under carefully controlled conditions. The amount of template RNA used for cDNA probe synthesis was adjusted to the same level according to the reported poly(A)$^+$ RNA content of the embryos (Clegg & Pikó, 1983*a*), and the conditions of hybridization and autoradiography were identical. The intensity of hybridization of the individual clones was measured by densitometric scanning of autoradiographs exposed for varying lengths of time. These data, together with readings obtained for several standard clones that were included in these experiments, were used for a quantitative evaluation of the developmental changes observed in a number of the cloned sequences, and are discussed in detail below. One or more additional cDNA library screens were performed for each stage of development and those gave a similar general pattern of reactivity but are not included in the quantitative data.

Reactivity of standard clones

The hybridization experiments shown in Fig. 1 included four clones representing sequences whose transcript prevalence was independently determined or could be derived with some measure of accuracy from available data: the mRNAs for β-actin and γ-actin; COI plus COII mRNAs (the plasmid clone contained the sequences of both mRNAs), and IAP RNA (Table 1). The abundance of the mRNAs for β- and γ-actin was derived from the reported data for total actin mRNA (Giebelhaus *et al.* 1985) and the relative amounts of these two sequences as determined in the present study from their reactivity with the cDNA probes used (see Footnote to Table 1). The ratio of γ-actin mRNA to β-actin mRNA increases significantly during development, from about 2·5 % in the egg to 5 % in 2-cell embryos and about 16–18 % in 8-cell embryos and early blastocysts. The 3′ untranslated segments of the human β- and γ-actin mRNAs, from which the clones used in the present study were derived, share a high degree of sequence homology with the corresponding segments of rodent actin mRNAs (Ponte *et al.* 1983; Erba, Gunning & Kedes, 1986; Tokunaga *et al.* 1986) and would be expected to be efficiently detected in the hybridization conditions used. The relationship between the densitometric tracings of the standard clones and their relative abundance in total embryo poly(A)$^+$ RNA is illustrated in Fig. 2. The best fit curve to these data has been used as a standard to estimate the relative abundance of the unknown clones. A reaction was considered positive at a density reading of 0·6 which corresponds to a sequence abundance of about 0·01 %, or about 700 to 3400 molecules per embryo depending on the stage of development. Suggestive positive readings, which were frequently

Table 1. *Abundance of standard RNA transcripts in poly(A)$^+$ RNA from early mouse embryos**

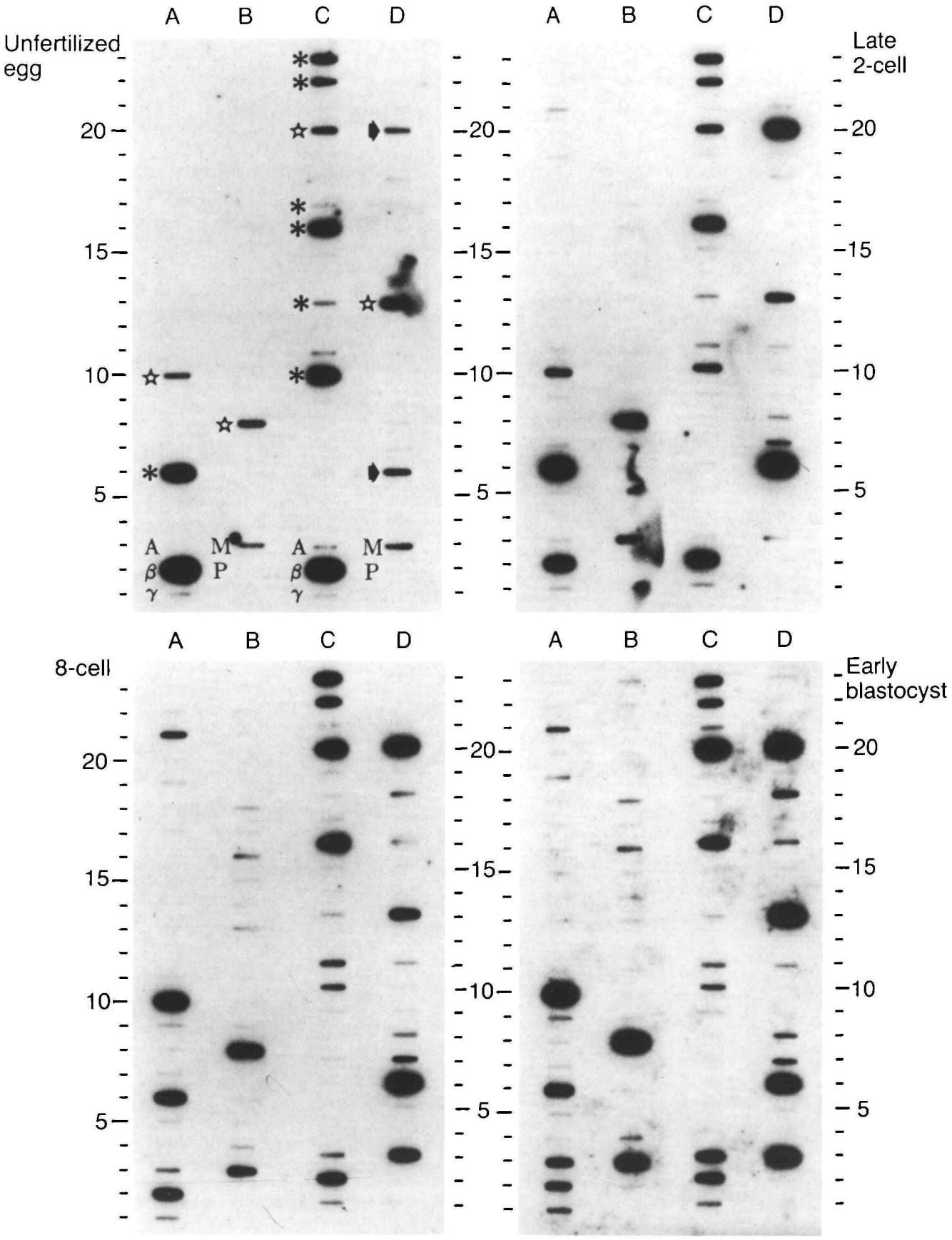
Stage of development	β-actin mRNA†		γ-actin mRNA†		Intracisternal A-particle RNA‡		COI+COII mRNA§	
	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)
Unfertilized egg	$3{\cdot}6\times10^5$	2·12	$9{\cdot}1\times10^3$	0·05	$1{\cdot}3\times10^3$	0·01	$3{\cdot}5\times10^4$	0·21
Late 2-cell	$2{\cdot}8\times10^4$	0·40	$1{\cdot}4\times10^3$	0·02	$7{\cdot}1\times10^3$	0·10	$2{\cdot}4\times10^4$	0·34
8-cell	$1{\cdot}2\times10^5$	0·94	$1{\cdot}9\times10^4$	0·14	$9{\cdot}7\times10^3$	0·08	$2{\cdot}1\times10^5$	1·61
Early blastocyst	$5{\cdot}5\times10^5$	1·62	$9{\cdot}9\times10^4$	0·29	$3{\cdot}8\times10^4$	0·12	$1{\cdot}2\times10^6$	3·53

* The %-abundance is calculated in relation to the number of total poly(A)$^+$ RNA molecules reported by Clegg & Pikó (1983*a*): unfertilized eggs, $1{\cdot}7\times10^7$; late 2-cell, $0{\cdot}7\times10^7$; 8-cell, $1{\cdot}3\times10^7$; early blastocyst, $3{\cdot}4\times10^7$.

† For β-actin and γ-actin mRNA, the number of molecules was calculated from the data of Giebelhaus *et al.* (1985) for total actin mRNA and the relative fraction of each of these mRNAs as determined in this study from the slot densities of the corresponding clones. The fractions were derived by dividing the measured density (D) of each mRNA clone with the total density expected for all actin mRNAs, the latter being calculated from the formula: $D_{total} = D_\beta + 2D_\gamma$. The value of D$\gamma$ was multiplied by 2, since the protein synthetic pattern suggests that a similar amount of α-actin mRNA is also present at least from the 8-cell stage onward (Abreu & Brinster, 1978).

‡ From Pikó *et al.* 1984.

§ Combined amount of the mRNAs for cytochrome *c* oxidase subunits I and II (from Pikó & Taylor, 1987).


A
B
C
D
Unfertilized egg
Late 2-cell
8-cell
Early blastocyst
20
15
10
5
A
β
γ
M
P

confirmed by higher readings at successive stages of development, were obtained at slot densities as low as 0·2. In general, there was no measurable reaction with pUC DNA used as background control.

Developmental patterns of transcript prevalence

Fig. 3 summarizes the overall patterns of the prevalence of the cloned sequences in early embryos. Of the 69 embryo-derived clones included in Fig. 1, 43 gave a positive reaction, defined as a density reading of 0·6 or higher, at least at some stage of development. In drawing individual curves, lower readings were also included when corroborated by positive readings at adjacent developmental stages. Six of the clones represented highly abundant transcripts (shown by heavier solid lines in Fig. 3); these were subsequently identified as members of the B1 and B2 repeat families and are discussed in greater detail below. The remaining 37 positive clones apparently represent rare to moderately abundant sequences, with transcript numbers varying from a few hundred to tens of thousands per embryo depending, in part, on the stage of embryo development.

The 37 positive clones can be subdivided broadly into two groups. 17 clones gave a detectable reaction at all four stages examined, including the egg (these clones are represented by broken lines in Fig. 3). In contrast, the remaining 20 clones were not detected at the 1-cell stage, but gave a clearly positive reaction with cDNA derived from 8-cell and blastocyst-stage embryos, and all but two of them reacted also, albeit sometimes only marginally, with cDNA from 2-cell embryos (represented by light solid lines in Fig. 3). The absence of reactivity at the 1-cell stage does not necessarily mean that these sequences are absent in the egg but only that their relative abundance is reduced as compared with the 2-cell stage. Since the total poly(A)$^+$ RNA content of the egg is about three times higher than that of the 2-cell embryo (Clegg & Pikó, 1983*a*), a transcript which is present in the same copy number at each stage would be diluted threefold in the egg as compared with the 2-cell stage and could remain undetected. The two groups of transcripts seem to differ in their overall abundance in the 2-cell

Fig. 1. Dot hybridization screening of 2-cell-stage cDNA library, showing the developmental pattern of expression of the cloned sequences. Plasmid DNA from 76 cDNA clones, which were found nonreactive with various other probes in preliminary screenings (see text), were dotted onto nitrocellulose filters and hybridized with ^{32}P-cDNA (specific activity about 2×10^9 cts min^{-1} μg^{-1}; total radioactivity about 1×10^7 cts min^{-1} ml^{-1}) transcribed from carrier-free RNA from unfertilized eggs, late 2-cell embryos, 8-cell embryos, and early blastocysts. The autoradiographs shown were exposed for 65 h. The bottom three rows of each filter contain duplicate dots of several standard clones and controls (see key below); slots B1 and D1 were left empty. Key: asterisk, clones identified as derived from the AMV reverse transcriptase preparation (A6, C10, C13, C17, C22, C23); arrow, B1 repeat sequences (D6, D20); star, B2 repeat sequences (A10, B8, C20, D18); β, human β-actin 3′-UTR clone (A2, C2); γ, human γ-actin 3′-UTR clone (A1, C1); A, IAP gene 3′ fragment (A3, C3); M, mitochondrial COI plus COII genes (B3, D3); P, pUC8 plasmid DNA (B2, D2).

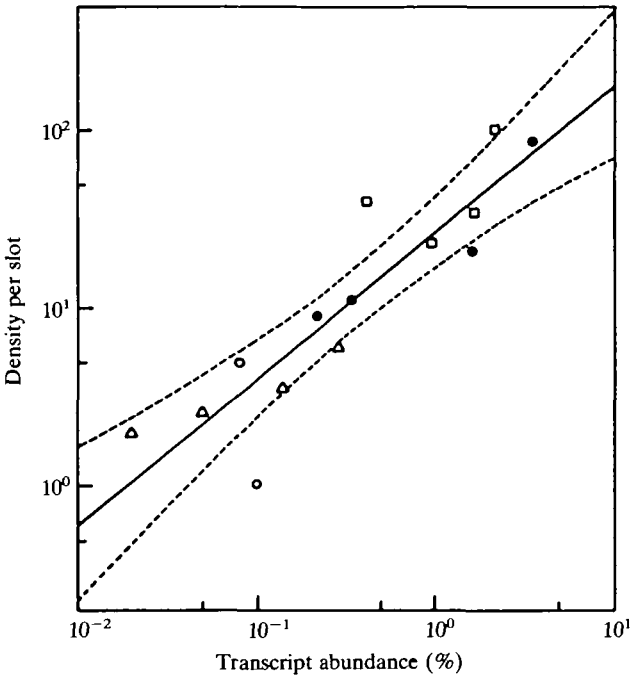


Fig. 2. Standard curve of slot density versus transcript abundance, obtained with clones complementary to transcripts of known abundance. The %-abundance of the mRNAs for β-actin, γ-actin, cytochrome *c* oxidase subunit I plus subunit II (COI and COII), and intracisternal A-particle (IAP) RNA (see Table 1) was plotted on a log–log scale against the density (in arbitrary units) of the corresponding slots in the experiment shown in Fig. 1. The plot incorporates the data obtained for all four stages of development, with the following exceptions. For IAP RNA, the positive reaction observed with unfertilized egg cDNA (grid C3) is not included because the duplicate slot was negative and two other similar hybridization experiments were also negative. The reactions obtained for IAP RNA with early blastocyst cDNA were also excluded because they were about eight times higher than the expected value; the reason for this discrepancy is not clear. The curve is the best fit line calculated by least-squares linear regression analysis. The data plotted fit the line with a coefficient of correlation, r^2, of 0·800. The broken lines indicate the 95 % confidence interval of the regression line (Dixon & Massey, 1969); the average interval is about $\pm1{\cdot}5\times$ in the middle register and about $\pm2{\cdot}2\times$ in the outer registers. Key: (□) β-actin mRNA; (△) γ-actin mRNA; (○) IAP RNA; and (●) COI + COII mRNAs.

embryo. Those clones that are first detected at the 2-cell stage are present, for the most part, at a very low abundance at this stage, less than 0·01 % (or about 700 molecules per embryo), and appear to represent rare transcripts. On the other hand, most of the clones that are also detected in the egg are present at an abundance of 0·01 % or higher at the 2-cell stage and clearly represent relatively more prevalent transcripts overall. One exception in the first group is clone A21 which is among the most abundant sequences from the 2-cell stage onwards but was not seen in the egg. Another cloned sequence, D16, is relatively abundant in the 8-cell embryo and the blastocyst but was not detected at the earlier stages.

An analysis of the developmental changes in transcript prevalence indicates that during development from the egg to the late 2-cell stage, transcript prevalence remains about the same or increases only moderately for most of the clones that have reacted at the 1-cell stage; nevertheless, due to the drop in the number of total poly(A)$^+$ RNA molecules between the egg and the 2-cell embryo (Clegg & Pikó, 1983*a*), the number of transcripts generally declines, on an average to about one-half, for the majority of the

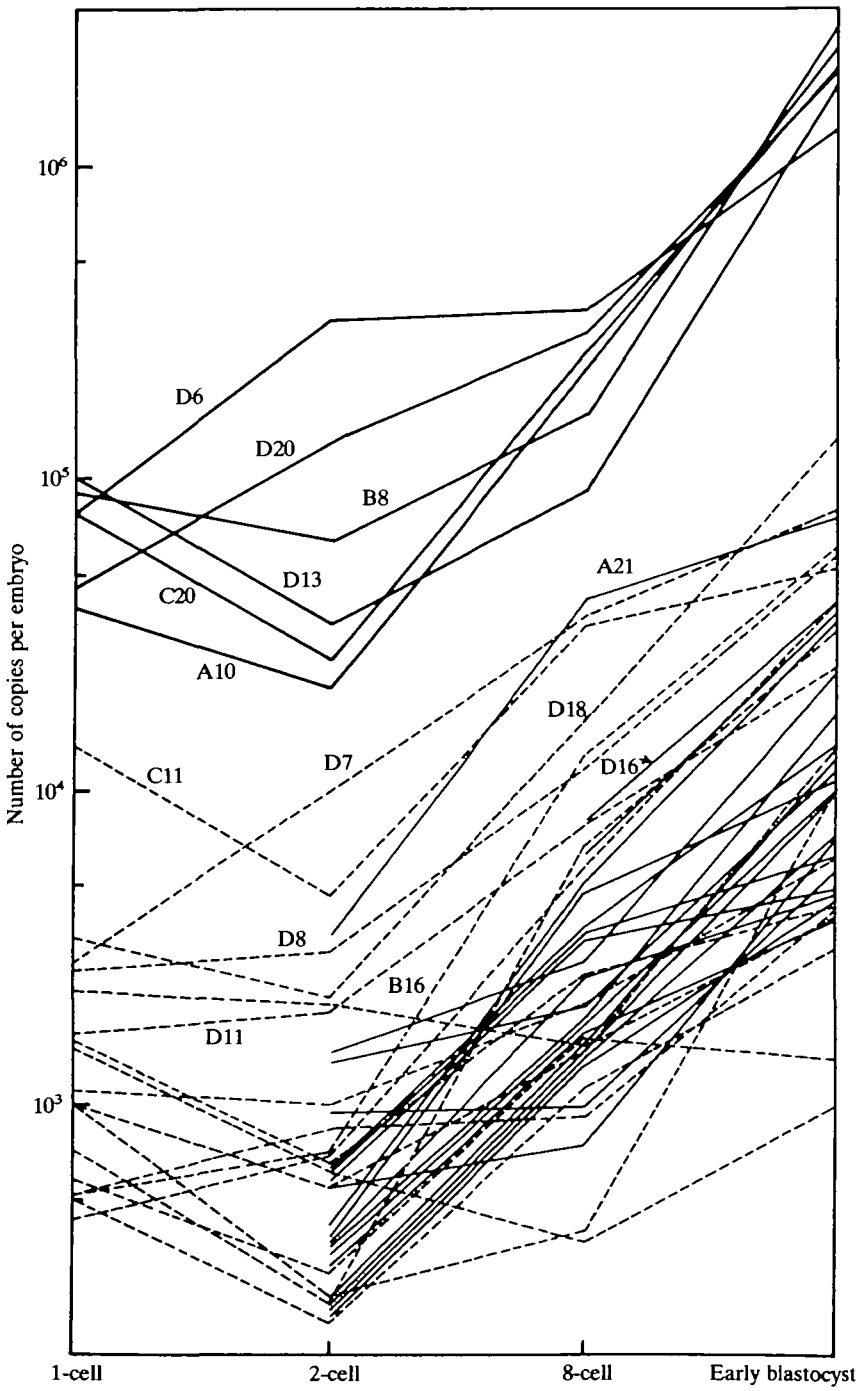


Fig. 3. Semilogarithmic plot showing the changes in the prevalence of individual cloned transcripts at different stages of development. Slot densities were determined by densitometric tracings of the slots obtained in the experiment shown in Fig. 1 and the relative abundance of each reactive clone was derived from the standard curve (Fig. 2). The number of copies per embryo was calculated from the percent abundance of the transcript at each stage of development and the reported number of total poly(A)$^+$ RNA molecules at that particular stage (Clegg & Pikó, 1983*a*; see also Footnote to Table 1). Broken lines represent clones that have given a detectable reaction at all four stages of development; light solid lines depict clones that were nonreactive at the 1-cell stage but gave a positive reaction at the 2-cell and later stages; and heavier solid lines show six highly abundant clones belonging to the B1 and B2 repeat families (see Table 2). The grid positions of some of the more abundant clones are indicated.

cloned sequences. A different pattern is exhibited by clone D7, representing one of the more abundant transcripts: this clone increases about ten times in abundance and four times in copy number between the egg and the 2-cell stage.

The most dominant developmental change observed in this study is the consistent rise in abundance and in copy number per embryo of nearly all of the clones from the 2-cell stage onwards through the blastocyst stage. The major part of the increase in relative abundance, on an average about three times, occurs between the 2-cell and 8-cell stages, with little or no further increase at the blastocyst stage. However, since the total poly(A)$^+$ RNA content increases about five times between the 2-cell stage and the early blastocyst stage, the number of copies per embryo increases by an average of about 15 times during the same period. For most of the clones, the increase in copy number falls within a range of from 5 to 25 times, but for a few clones the rise is as high as 50–100 times (for example, clones B16 and D18). The overall increase in abundance from the 2-cell stage onward is similar for all clones, regardless whether they have been detected at the 1-cell stage or not, indicating an active synthesis and accumulation of both types of transcripts during the cleavage stages.

Fig. 4 illustrates the distribution of transcript prevalence for 63 of the 69 2-cell cDNA clones (clones representing B1 and B2 sequences are excluded) in 2-cell, 8-cell and early-blastocyst-stage embryos. The fraction of clones falling into a given prevalence class is expected to reflect the mass fraction of poly(A)$^+$ RNA of that prevalence in the embryo (Lasky *et al.* 1980). The results indicate that, on a per cell basis, the relative distribution of the cloned sequences among the various (arbitrary) prevalence classes is similar at these three stages, with 40 to 50% of the clones representing very infrequent transcripts (<100 copies per cell). Low prevalence (complex class) mRNA sequences constitute about half of the mRNA mass also in the embryos of several invertebrate species (Davidson, 1986). From these data and the total number of poly(A)$^+$ RNA molecules at each stage of development, one can estimate the approximate number of different mRNA species per embryo in the more abundant prevalence classes (indicated by the numbers above the histogram columns in Fig. 4). Although the numbers of different transcripts falling into the least abundant sequence class cannot be estimated with any accuracy, a rough extrapolation from the data in Fig. 4 suggests an overall mRNA population on the order of 10^4 diverse species during the cleavage stages.

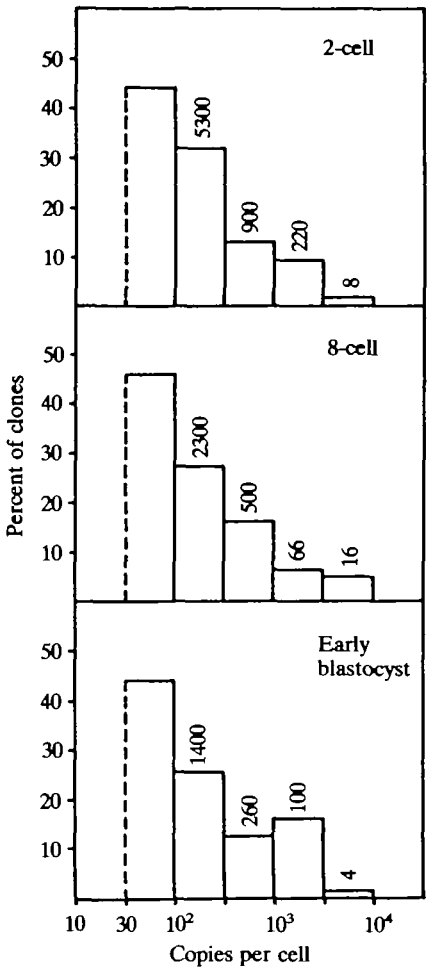


Fig. 4. Histograms showing the prevalence distribution of transcripts complementary to the 2-cell mouse cDNA library. The average number of copies per cell was calculated from the number of copies per embryo obtained as described in Fig. 3 and the number of cells per embryo; early blastocysts were considered to have an average of 32 cells. Only the 63 clones not representing B1 and B2 repeats are included in the histograms. The numbers above each column in the histogram indicate the approximate number of different mRNA species per embryo calculated from the formula Nf/c, where N is the total number of poly(A)$^+$ RNA at each stage of development (from Clegg & Pikó, 1983*a*; see Footnote to Table 1); f is the fraction of the clones falling in that class; and c is the mean number of copies per embryo represented by that class (Lasky *et al.* 1980). No complexity calculation was made for the very rare sequence class (<100 copies per cell) which consists of marginally reactive clones whose abundance could not be reliably estimated.

High prevalence of B1 and B2 transcripts

Among the 69 embryo-derived clones, six clones represented highly abundant sequences (shown in the upper register of Fig. 3). Cross-hybridization experiments indicated that the six clones belonged to two homology groups, the members of which cross-

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                                                                                                    70
B1 consensus  CCGGGCGTGG TGGCGCACGC CTTTAATCCC AGCACTCGGG AGGCAGAGGC AGGCGGATTT CTGAGTTCGA
Clone D20                                     ....T.T... .......... ...T...... ...G...T..
Clone D6                                 .A.. ....T.TA.. .......... .......... ........A.
                                                                                       135
B1 consensus  -GGCCAGCCT GGTCTACAGA GTGAGTTCCA GGACAGCCAG GGCTACACAG AGAAACCCTG TCTNN
Clone D20     -..A...... .......... .......... .......... .......... .......... ...TG
Clone D6      A......... ...T....A. ..-..C...T ......A... .....T.T.. .......... ...GA


                                                                                                    70
B2 consensus  GGGGCTGGAG AGATGGCTCA GTGGTTAAGA GCACCTGACT GCTCTTCCGA AGGTCCTGAG TTCAATTCCC
Clone D13                            ..G..... .....C.... .......... ......A... .....A....
Clone B8                             ...G.T... .....C.... ........AG ......G... .....G....
Clone C20     C......... .......... .C....C... ...-...... ........A. .........A .....A....
                                                                                       140
B2 consensus  AGCAACCACA TGGTGGCTCA CAACCATCCG TAA--TGATC TGATGCCCTC TTCTGGAGTG TCTGAAGACA
Clone D13     .......... .......... ........T. ...CAA.... ...CA..... .......... ..........
Clone B8      .......... .......... ........T. ...CAA.... .......... ......G..A ..........
Clone C20     ..A....... ..........
                                         170
B2 consensus  GCTACAGTGT ACTTACATAT AATAAATAAA
Clone D13     .......... .......
Clone B8      ..........


                                                                                                    70
Clone C20     AAAAATTTTT TAAAAAGAAA GATTAAGGGT AGAGGGACTG CATGTAATGA GCCTGAGACC CTTTCTTGAC
                                                                                                   140
              CTGTAAGGCC TCATCAAGGT ATAGACAGTG TAGCTCTTCA TTGAATCAAG GTTCTTTGTG AATGCCAAGG
                                                                                                   210
              TCTAGCGTCC ATGGAGGCAT CTTAGATGAA CAAAACAATG CTCTCCTTAC AAAGAAATAA CGGGCTGGAG
                                                                                                   280
              AGATGGCTCA GCGGTTCAGA GCACTGACTG CTCTTCCAAA GGTCCTGAAT TCAAATCCCA GAAACCACAT

              GGTGGCTCA
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Fig. 5. Sequence comparison of the B1 and B2 clones isolated from the mouse 2-cell cDNA library with the consensus sequences for B1 (Kalb *et al.* 1983) and B2 elements (Krayev *et al.* 1982). Only nucleotides that are different from the consensus sequence are indicated. Dashes indicate gaps introduced to obtain an optimal alignment. The complete sequence of the insert in clone C20 including a 200-nucleotide flanking segment is also shown (the B2 sequence is underlined).

hybridized extensively. Subsequent nucleotide sequencing identified two of the clones (D6 and D20; marked with arrows in Fig. 1) as members of the B1 repeat family and four of the clones as containing B2 repeat sequences (A10, B8, C20 and D13; marked with stars in Fig. 1).

Fig. 5 shows the nucleotide sequences of the B1 and B2 clones and their homologies (ranging from 87 to 94%) to the B1 and B2 consensus sequences. Clone A10 had a sequence identical to that of Clone B8; we assume that these two clones were inadvertently duplicated during isolation. The clones contained no other sequence in addition to (incomplete) copies of the B1 or B2 repeat sequence, except for clone C20 which included a stretch of 200 nucleotides adjacent to the 5′ region of the B2 consensus sequence (Fig. 5, bottom). The flanking sequence contains several stop codons in all reading frames in both orientations; a search of the EMBL gene library turned up no extensive homology of this segment to any known sequence. We have not tested the reactivity of the 200-nucleotide segment with embryo-derived cDNA probes but we assume that the high reactivity of clone C20 with these probes is due to the presence of the B2 repeat sequence.

The developmental pattern of prevalence of B1 and B2 transcripts is shown diagrammatically in Fig. 3 and the data on the relative abundance and number of these transcripts are summarized in Table 2. The transcripts of both repeats are similarly abundant in the egg, with copy numbers of about $6–8\times10^4$ each. However, the expression of the two repeats appears to differ sharply at the 2-cell stage: there is approximately a tenfold increase in the relative abundance and a fourfold increase in the copy number of B1 transcripts in the 2-cell embryo as compared with the egg, whereas the abundance of B2 transcripts increases only slightly and their copy number decreases

Table 2. *Abundance of B1 and B2 transcripts in relation to total poly(A)$^+$ RNA in early mouse embryos*

Stage of development	B1 transcripts		B2 transcripts	
	(%)	(No.)	(%)	(No.)
Unfertilized egg	0·35 ± 0·09	$6·0\times10^4$	0·45 ± 0·08	$7·6\times10^4$
Late 2-cell	3·22 ± 1·38	$2·2\times10^5$	0·52 ± 0·13	$3·6\times10^4$
8-cell	2·33 ± 0·18	$3·0\times10^5$	1·39 ± 0·27	$1·8\times10^5$
Early blastocyst	4·81 ± 1·10	$1·6\times10^6$	6·32 ± 0·67	$2·1\times10^6$

The %-abundance values ±S.E.M. are derived from the slot densities of the two B1 clones and the four B2 clones in the experiment shown in Fig. 1 and the standard curve shown in Fig. 2. The number of molecules is calculated from the %-abundance of these transcripts and the number of total poly(A)$^+$ RNA molecules (Clegg & Pikó, 1983*a*; see also Footnote to Table 1). The number of B1 transcripts is significantly different from that of B2 transcripts in the 2-cell embryo (Student's *t*-test; $P = 0·05$) but not at the other stages of development.

to about one half during the same period. On the other hand, the abundance of B2 transcripts rises more rapidly than that of the B1 transcripts during cleavage through the blastocyst stage and, as a consequence, both transcripts reach similar copy numbers by the early blastocyst stage. In early blastocysts, the steady-state amounts of B1 and B2 transcripts are estimated at about 2×10^6 copies per embryo each, or about 5–6 % of the total number of poly(A)$^+$ RNA at this stage (Table 2).

Discussion

Preliminary screening of cDNA library

In this work, the original cDNA library was synthesized from about 5 ng of poly(A)$^+$ RNA from late 2-cell embryos in the presence of an approximately 1000 times excess of MS2 bacteriophage RNA carrier. Subsequent screening of the transformant colonies and of isolated plasmid DNA revealed a heavy representation of MS2-derived sequences in the cDNA library (about 70 % of the total clones), with minor fractions containing *E. coli* DNA fragments (about 3 %) and sequences derived from the AMV reverse transcriptase preparation (about 2 %). The *E. coli* DNA could have contaminated the MS2 RNA preparation or the various *E. coli*-derived enzymes used in cDNA synthesis and tailing. The presence of AMV-derived clones is noteworthy because these clones reacted strongly when screened with cDNA synthesized with the same enzyme preparation. It is reasonable to assume that the remaining 25 % of the clones represent embryo RNA because no other source of contamination could be identified and the majority of the clones reacted in a stage-specific fashion when hybridized with cDNA probes obtained from embryos at different stages of development. One of the 70 embryo-derived clones was shown to contain mitochondrial sequences and was omitted from further screening; this low frequency is in agreement with the estimated mitochondrial mRNA content, about 2 %, of the total poly(A)$^+$ RNA at the 2-cell stage (Pikó & Taylor, 1987). Although a total poly(A)$^+$ RNA preparation was used for cDNA synthesis, it is likely that most of the remaining clones have originated from cytoplasmic poly(A)$^+$ RNA and that they represent mRNA sequences. It can be estimated from *in situ* hybridization data with ^{3}H-poly(U) that 10–15 % of the total poly(A) at the late 2-cell stage is localized in the nuclei while the remainder is cytoplasmic (Pikó & Clegg, 1982; and unpublished observations).

Developmental patterns of transcript prevalence

The relative abundance of transcripts complementary to the selected cDNA clones in poly(A)$^+$ RNA from embryos at different stages of development was estimated in dot hybridization experiments where filter-bound cloned DNA was in large sequence excess over the labelled cDNA probes. In these conditions, the amount of radioactivity bound per dot is primarily a function of the concentration of the cloned sequence in the cDNA probe which, in turn, reflects the prevalence of complementary transcripts in the template RNA (Kafatos, Jones & Efstratiadis, 1979; Lasky *et al.* 1980). This expectation has been corroborated by the reactivity of several standard clones: the autoradiographic density of the dots of these clones shows a good agreement, generally within a factor of two, with the relative abundance of the corresponding transcripts derived from independent experiments. The best-fit curve obtained with the standard clones (Fig. 2) was used to calculate the relative abundance of the unknown clones without any correction for insert size. In most cases, the variation in insert size would not have had a greater than twofold effect on the abundance values, except possibly for B1 and B2 transcripts whose cloned sequences were on an average about one-fourth those of the standards and, therefore, their abundances may have been underestimated by as much as a factor of four. In any case, the changes in prevalence

observed between different developmental stages for the same clone are unaffected by the size of the insert.

Among the standard clones, the results provide some new information on the expression of actin mRNAs in the early mouse embryo. They confirm the presence of a large store of actin mRNA in the egg, as reported by Giebelhaus *et al.* (1985), and indicate that the bulk of this mRNA codes for β-actin but that a small amount of γ-actin mRNA is also present. Although the maternally derived actin mRNA becomes deadenylated during oocyte maturation (Bachvarova *et al.* 1985), a short stretch of poly(A) tail apparently remains since the actin mRNA of the egg is efficiently transcribed in an oligo(dT)-primed reverse transcriptase reaction. During cleavage, the ratio of γ-actin to β-actin mRNA increases from about 2·5% in the egg to about 18% in early blastocysts, suggesting that the accumulation of the two mRNAs is regulated independently during early development. A differential expression of the β- and γ-actin genes was observed in cultured chicken cells treated with phorbol esters (Gerstenfeld, Finer & Boedtker, 1985) and in various human tissues and cell lines (Chou *et al.* 1987). In the sea urchin embryo, four cytoskeletal actin genes are expressed and show distinct patterns of transcript accumulation (Lee *et al.* 1986). Whether, in the mouse embryo, cytoskeletal actin genes other than those coding for the β- and γ-actin isotypes are active is not known but the present results make it unlikely that they would account for a large fraction of the total actin mRNA. However, the protein synthetic pattern suggests that a small amount of α-actin is expressed in mice at least from the 8-cell stage onward (Abreu & Brinster, 1978).

A major result of this study is that about one half of the clones that gave a positive reaction at the 2-cell and later stages did not react detectably with cDNA probes from the egg, suggesting that their transcripts are absent or very rare in egg poly(A)$^+$ RNA but accumulate from the 2-cell stage onward as a result of new transcription from the embryonic genome. An alternative possibility is that these transcripts lack poly(A) in the egg (and therefore do not contribute to the cDNA probe used for screening) but are present in a polyadenylated form at later stages of development either as a result of new synthesis of the complete poly(A)$^+$ RNA or cytoplasmic polyadenylation of the stored maternal mRNA. Although cytoplasmic polyadenylation of previously nonpolyadenylated RNA takes place in the fertilized egg and 2-cell embryo (Clegg & Pikó, 1983*a*,*b*), it is unlikely to be significantly involved in the qualitative shift in the mRNA population occurring during the 2-cell stage for several reasons. (1) The appearance of many new transcripts at the late 2-cell stage is in agreement with the major shift in the qualitative pattern of newly synthesized embryonic proteins which occurs at this time (see Introduction). The establishment of the new protein pattern is sensitive to inhibition by α-amanitin, suggesting that it is dependent on new transcription by the embryonic genome (Flach *et al.* 1982; Bensaude *et al.* 1983; Bolton *et al.* 1984). (2) *In vitro* translation experiments in a cell-free system using total RNA from very early *versus* late 2-cell embryos as template suggest that the mRNA populations of these two stages are qualitatively quite different. Furthermore, RNA extracted from late 2-cell embryos that had been incubated during the preceding 20 h in the presence of α-amanitin shows no *in vitro* template activity suggesting the degradation of most or all of the maternally inherited mRNAs (Bolton *et al.* 1984). (3) The steep drop in poly(A) content, to about one third, between the 1-cell and late 2-cell stage (Pikó & Clegg, 1982) and the active synthesis of poly(A)$^+$ RNA from the 2-cell stage onward (Levey *et al.* 1978; Clegg & Pikó, 1983*a*,*b*) are consistent with the degradation of most of the maternal mRNA and its replacement by newly synthesized mRNA derived from the embryonic genome. The levels of some individual mRNAs derived from maternally active genes, namely, those coding for actin and several histone proteins, show a similar developmental pattern but with an even sharper decline (about 10×) in the 2-cell embryo (Giebelhaus *et al.* 1983, 1985; Graves *et al.* 1985).

It is reasonable to conclude from the above considerations that the full transcriptional activation of the embryonic genome at the 2-cell stage is a major regulatory event which results in the production, in addition to maternal type gene transcripts, also of many new mRNA species that are not represented in significant amounts in egg RNA. The results of the present study suggest that most of the transcripts that first appear at the 2-cell stage continue to be synthesized and increase considerably both in abundance and copy number per embryo during the succeeding cleavage stages. The increase in relative abundance occurs primarily between the 2-cell and 8-cell stages, which is consistent with the observation that all major qualitative changes in the pattern of preimplantation protein synthesis take place by the 8-cell stage (Van Blerkom & Brockway, 1975; Levinson *et al.* 1978).

The approximately equal representation of transcripts derived from maternally active genes *versus* transcripts derived from zygote-specific genes in the 2-cell mouse cDNA library is in sharp contrast to the quantitative dominance of the maternal gene set in the early embryos of several invertebrate and lower vertebrate species. For example, in the sea urchin embryo, it has been estimated that about 90% of the mRNA sequences expressed up to the pluteus stage are represented also by transcripts of comparable

prevalence in maternal RNA stored in the egg (Flytzanis, Brandhorst, Britten & Davidson, 1982; Davidson, 1986). A similarly low frequency of embryo-specific transcripts was found in cDNA libraries from *Xenopus* gastrulae (Dworkin & Dawid, 1980; Dawid *et al.* 1985) and *Drosophila* blastoderm-stage embryos (Roark, Mahoney, Graham & Lengyel, 1985; see general discussion by Davidson, 1986). The reason for this difference between the mouse and nonmammalian species is not clear but it could be related to the much slower pace of embryo development in the mammal and the need for the differentiation of structures involved in implantation. There is evidence suggesting that about one half the polyadenylated RNA stored in the full-grown mouse oocyte is deadenylated and/or degraded during the 12 h of meiotic maturation preceding fertilization (Bachvarova *et al.* 1985). Other features of early mouse embryogenesis that differ from the sea urchin and amphibian model include the early activation of ribosomal RNA synthesis which begins at the 2-cell stage (Knowland & Graham, 1972; Clegg & Pikó, 1982), the five- to sixfold increase in the content of total RNA and mRNA between the 2-cell and early blastocyst stages (Levey *et al.* 1978; Pikó & Clegg, 1982; Clegg & Pikó, 1983*a*), and the apparently programmed elimination of most of the remaining maternal mRNAs between the early and late 2-cell stage (discussed above). It appears that a nearly complete transition from maternal to embryonic control of development occurs at the 2-cell stage and that this transition involves the activation of a large set of zygotic genes that were either inactive during oogenesis or did not contribute significant amounts of stable transcripts to the maternal mRNA set stored in the egg. An example of the latter possibility is the pattern of prevalence of RNA derived from intracisternal A-particle genes: in this case, the number of transcripts is relatively high in ovarian oocytes but is reduced to about 1/10th in the egg, followed by an approximately 100-fold increase during cleavage through the blastocyst stage (Pikó *et al.* 1984). Quantitative assays of transcript prevalence using cloned cDNA probes should be useful in identifying those genes that are silent during oogenesis and first become activated in the early embryo.

Expression of B1 and B2 transcripts

An interesting observation in this study is the abundant expression of B1 and B2 transcripts in the early mouse embryo. The B1 and B2 sequences are short, interspersed, repetitive elements which are present in about 10^5 copies each in the haploid mouse genome (Krayev *et al.* 1982). B1 repeats are about 130 bp in length and share extensive sequence homology with the monomeric unit of the 300 bp human Alu sequence (Schmid & Jelinek, 1982). B2 repeats are about 180 bp long and have a number of structural features in common with B1 repeats, such as an A-rich 3′ region, RNA polymerase III split promoter sites and flanking direct repeat sequences on either side; however, B1 and B2 repeats share only a limited sequence homology with each other (Jelinek & Schmid, 1982). Within each family, sequence variability is generally within 10% of the consensus sequence (Krayev *et al.* 1982; Kalb, Glasser, King & Lingrel, 1983; Kramerov, Tillib, Ryskov & Georgiev, 1985*b*).

B1 and B2 transcripts constitute about 2% of heterogeneous nuclear RNA (Krayev *et al.* 1982) but also occur interspersed in the 3′ untranslated segments of cytoplasmic mRNAs (Georgiev, 1984; Shuh *et al.* 1986) and as small, heterogeneous (100 to 500 nucleotides) cytoplasmic poly(A)$^+$ RNAs transcribed by RNA polymerase III (Schmid & Jelinek, 1982; Kramerov, Lekakh, Samarina & Ryskov, 1982; Kramerov *et al.* 1985*a*). The abundance of small B1 and B2 RNAs and the amounts and pattern of cytoplasmic mRNAs containing these repeats vary according to tissue: a greatly enhanced expression has been observed in many tumours (Georgiev, 1984; Grigoryan *et al.* 1985), virus-transformed (Scott, Westphal & Rigby, 1983; Singh, Carey, Saragosti & Botchan, 1985) as well as heat-shocked (Fornace & Mitchell, 1986) and growth-stimulated cells (Edwards, Parfett & Denhardt, 1985), early post-implantation embryos (Murphy *et al.* 1983), and also in embryonal carcinoma cells but not in their differentiated progeny (Murphy *et al.* 1983; Bennett *et al.* 1984).

In the preimplantation mouse embryo, Vasseur, Condamine & Duprey (1985) detected B2 transcripts in both the nuclei and cytoplasm by *in situ* hybridization: the abundance of cytoplasmic B2 transcripts (mostly or exclusively the canonical or plus strand sequence) increased throughout cleavage and, in blastocysts, was higher in the inner cell mass than in the trophectoderm; in 7·5-day embryos the expression of B2 sequences was restricted to ectoderm and mesoderm. Northern blot hybridization experiments indicate substantial amounts of small B1 and B2 transcripts (plus strand only) in mouse oocytes; smaller amounts of these sequences (both plus and minus strands) are found in mRNA-sized molecules (Kaplan, Jelinek & Bachvarova, 1985; Bachvarova & Paynton, 1986). The results of the present study corroborate and complement these findings, and indicate an active transcription of both B1 and B2 repeats during the cleavage stages. As measured by dot hybridization experiments of the cloned cDNAs with labelled cDNA probes, the abundance of both

B1 and B2 transcripts increases about 30-fold (from about 7×10^4 to about 2×10^6 molecules per embryo; Table 2) between fertilization and the blastocyst stage, but the pattern of increase is different for the two transcripts: for B1 transcripts the increase in copy number is greatest at the 2-cell stage whereas the abundance of B2 transcripts rises progressively during cleavage. This divergence suggests that the accumulation of the two transcripts is regulated independently. Although the molecular forms of the B1 and B2 RNAs detected in this study are not known, the structure of the cDNA clones obtained from the 2-cell embryo suggests that the majority are small, RNA polymerase III transcripts; however, one of the three B2 clones (clone C20; Fig. 5, bottom) has a B2 sequence interspersed in a larger molecule. The cDNA clones of the two repeats show a substantial sequence homology, generally better than 90 %, with the B1 and B2 consensus sequences (Fig. 5), but they also differ from each other to a similar extent, suggesting that the B1 and B2 transcripts in the embryo derive from a number of diverse genomic elements, as has been found for the small B2 RNAs of tumour cells (Kramerov *et al. 1985b*).

The role of the B1 and B2 repeats and their RNA transcripts is as yet unknown. There is evidence that small B1 and B2 RNAs are associated with mRNA by hydrogen bonding and are included in informosome-like complexes, thus possibly having a role in mRNA utilization (Kramerov *et al.* 1985*a*; Schoeniger & Jelinek, 1986). It has been proposed, on the basis of potential base-pairing interactions, that B2 repeats may regulate the stability of a subclass of transiently expressed mRNAs coding for proteins involved in cell proliferation (Clemens, 1987). More information on the mode of transcription, molecular forms and subcellular localization of B1 and B2 transcripts is needed before one can venture on their possible role in early embryonic development. Whatever their function may be, the apparent high level of transcription of B1 and B2 elements may prove useful in analysing some of the cellular factors involved in the transcriptional regulation of the zygote genome.

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